



3^ο ΣΧΟΛΕΙΟ ΒΑΣΙΚΗΣ ΑΝΟΣΟΛΟΓΙΑΣ ΓΙΑ ΚΛΙΝΙΚΟΥΣ

www.clinicalimmunology-crete-2021.gr

8-10 ΟΚΤΩΒΡΙΟΥ 2021

HOTEL IBIS STYLES HERAKLION CENTRAL
ΗΡΑΚΛΕΙΟ ΚΡΗΤΗΣ

ΗΡΑΚΛΕΙΟ ΚΡΗΤΗΣ
HOTEL IBIS STYLES HERAKLION CENTRAL
8-10 ΟΚΤΩΒΡΙΟΥ 2021

Mass cytometry

Κυτταρομετρία μάζας

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<https://www.brfaacytoflab.com/>

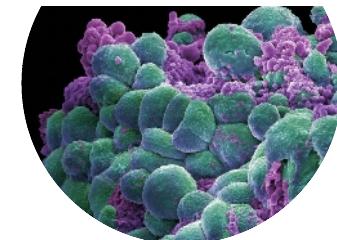
In memory of



- A bright scientist
- A passionate immunologist
- A cool person

Vily Panoutsakopoulou

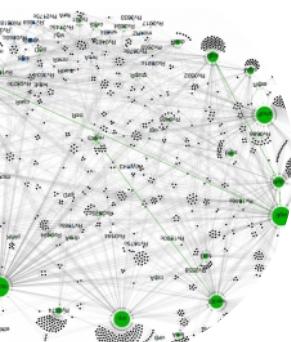
Ετερογένεια Βιολογικών συστημάτων → Παθοφυσιολογία ασθενειών
 Δείκτες για τον ορισμό κυτταρικών υποπληθυσμών
 (Cell Subsets)



Κάθε κύτταρο
είναι μοναδικό

B Cell T Cell Monocyte Dendritic Cell Eosinophil Neutrophil NK Cell ...

							...
CD19	CD3	CD11b	CD4	CD15	CD10	CD16	
CD20	CD4	CD11c	CD11b	CD23	CD11c	CD49b	
CD22	CD8	CD14	CD11c	CD49d	CD14	CD56	
CD44	CD25	CD15	CD49d	CD183	CD15	CD57	
CD45R	FOXP3	F4/80	CD80	CD294	CD16	CD69	
IgD	PD-1	HLA-DR	CD86	FcεR1α	CD62L		



Η πολυπλοκότητα αυτή χρειάζεται
 προηγμένα εργαλεία ανάλυσης στο
 επίπεδο μοναδιαίων κυττάρων
 (single cell analysis)

Δείκτες για τον ορισμό κυτταρικών
 λειτουργιών (Cell function)

Signaling/Transcription	Cytokines/ Growth Factors	Cell Death/ Apoptosis	Cell Cycle/ Proliferation
pNF-kB	IL-6		
p38	IL-10		
p4E-BP1	GM-CSF	IL-17A	Ki-67
pAkt	pSHP2	IFN-g	pH3
Nanog	pSLP-76	TNF-a	pRb
pERK1/2	pSTAT1	IL-2	
pLck	pSTAT3	IL-4	
Sox2	K.ά...	IL-5	

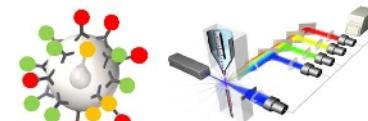
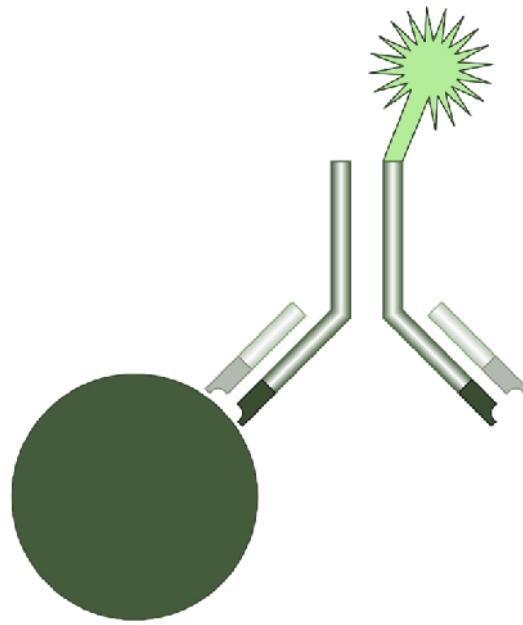
Heterogeneity in biology

Multiparameter cytometry
Colors and masses

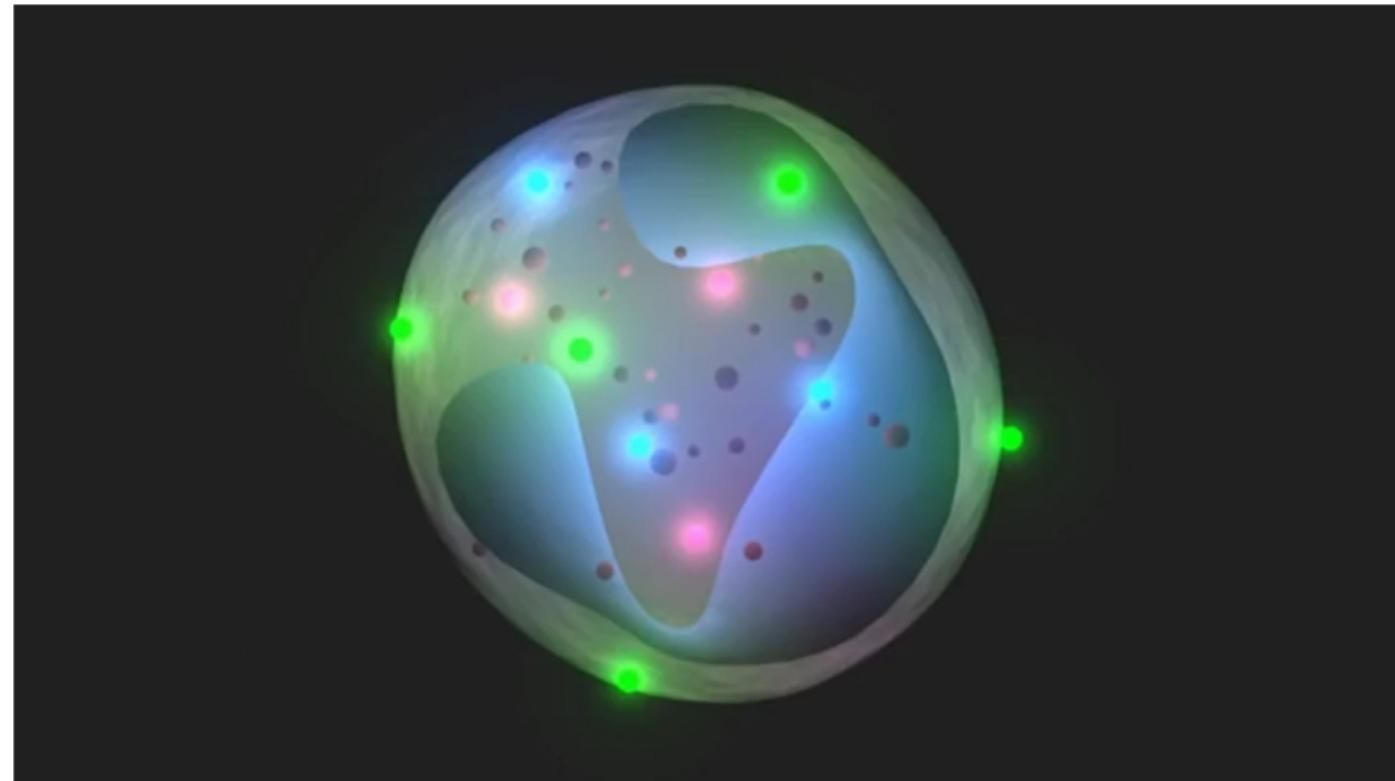
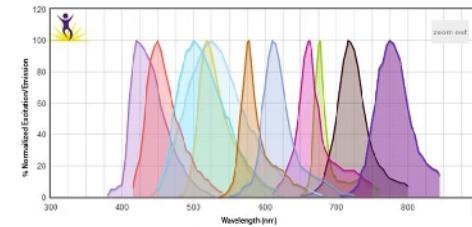
Multiparameter cytometry
CyTOF - Principles

Analysis of multiparametric
data in mass cytometry (CyTOF)

Single-cell analysis: Flow cytometry



Fluorophores: signal overlap
limits practical panel size



Heterogeneity in biology

Multiparameter cytometry >

Colors and masses

Multiparameter cytometry >

CyTOF - Principles

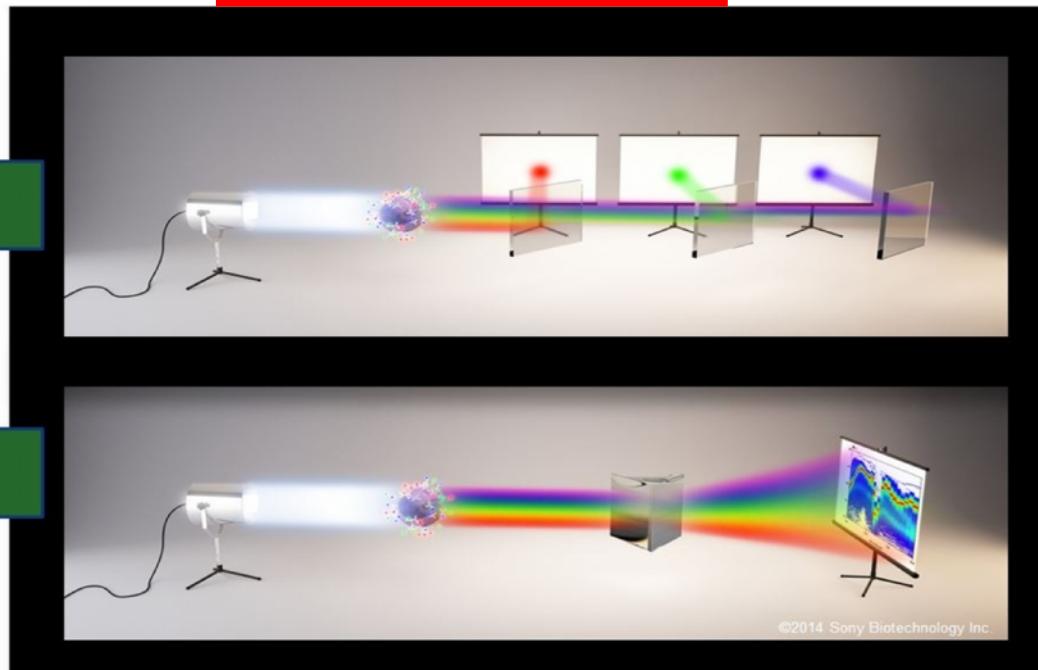
Analysis of multiparametric

data in mass cytometry (CyTOF) >

Conventional

Vs.

Spectral



ID7000™ Spectral Cell Analyzer

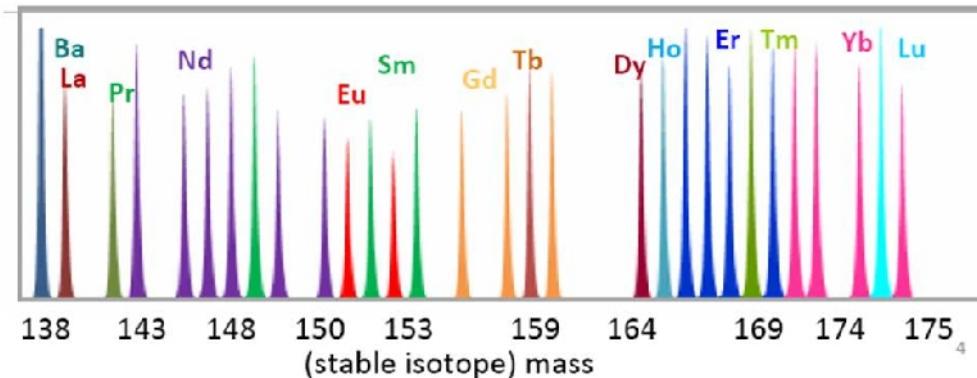
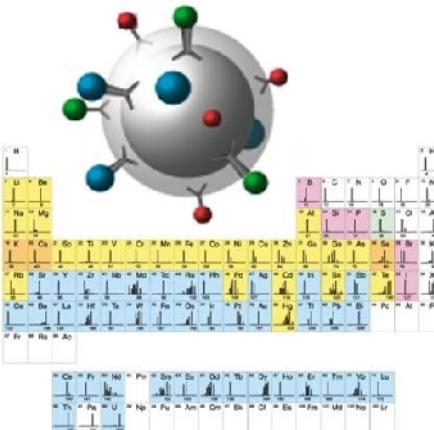
[Overview](#) [Features](#) [Specifications](#) [Resources](#)



A high-parameter spectral cytometer, the ID7000 delivers comprehensive information about heterogeneous cell populations, with high sensitivity to detect dim and rare populations.

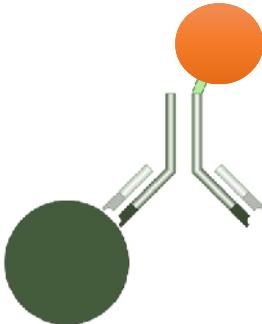
Expanding the boundaries of cell analysis, the ID7000 can be configured with up to 7 lasers and 186 detectors, the most of any flow cytometer available today. The system enables researchers to perform experiments using 44 colors, limited only by the fluorochromes available. The choice of lasers include blue (488 nm), red (637 nm), violet (405 nm), yellow-green (561 nm), ultraviolet (355 nm), deep ultraviolet (320 nm), and infrared (808 nm).

Cytometry for the masses..

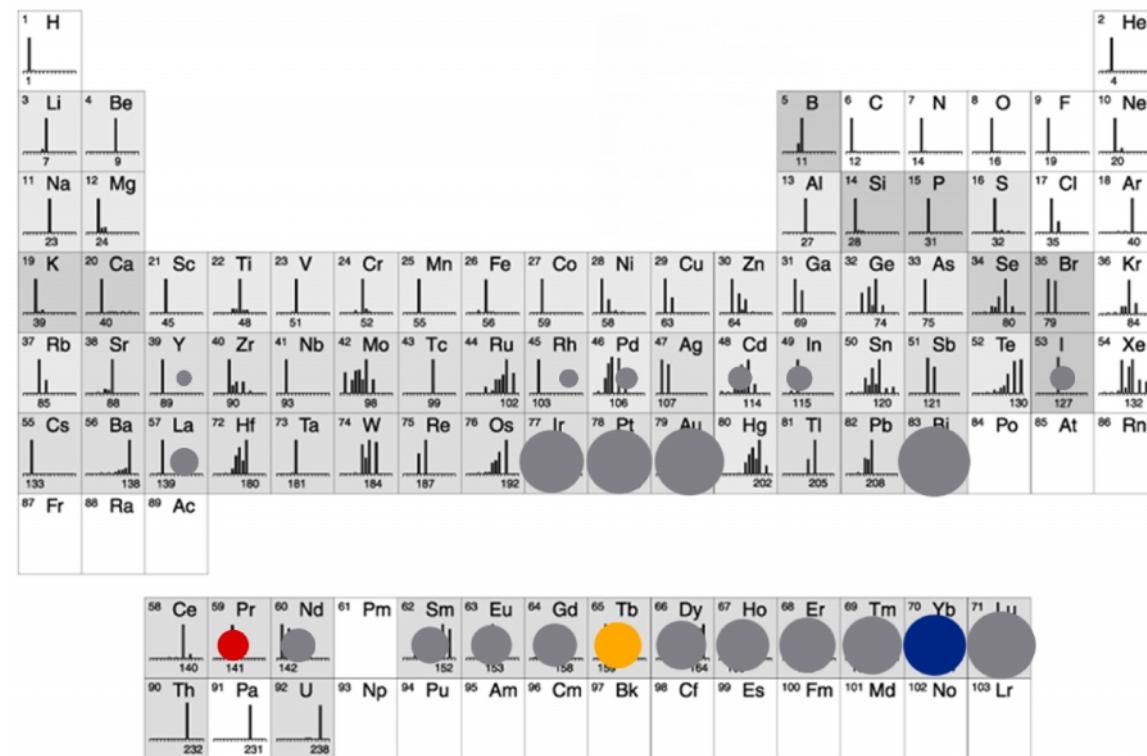


Κυτταρομετρία μάζας

Cytometry by Time-of-flight
(CyTOF)



Metals



high-purity metal isotope tags

Minimal background from signal overlap or endogenous cellular components

Heterogeneity in biology

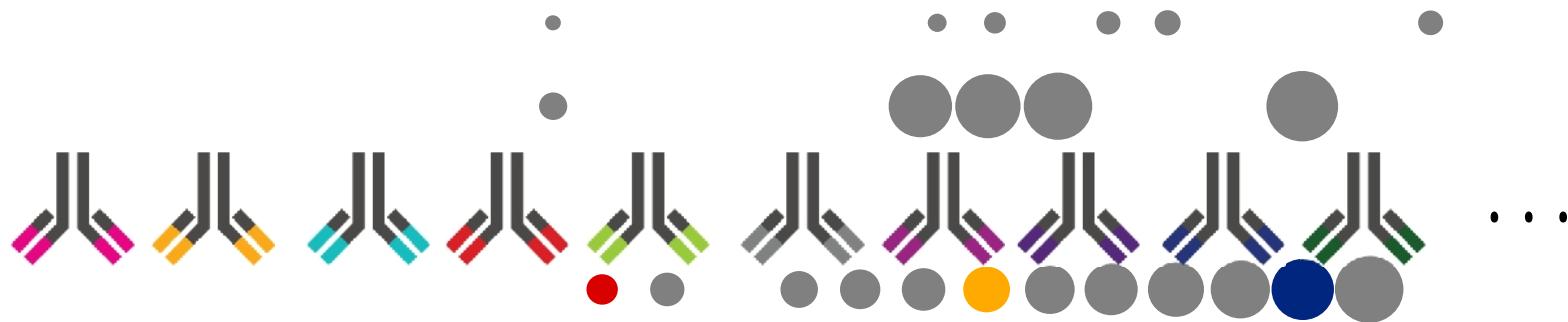
Multiparameter cytometry
Colors and masses

Multiparameter cytometry
CyTOF - Principles

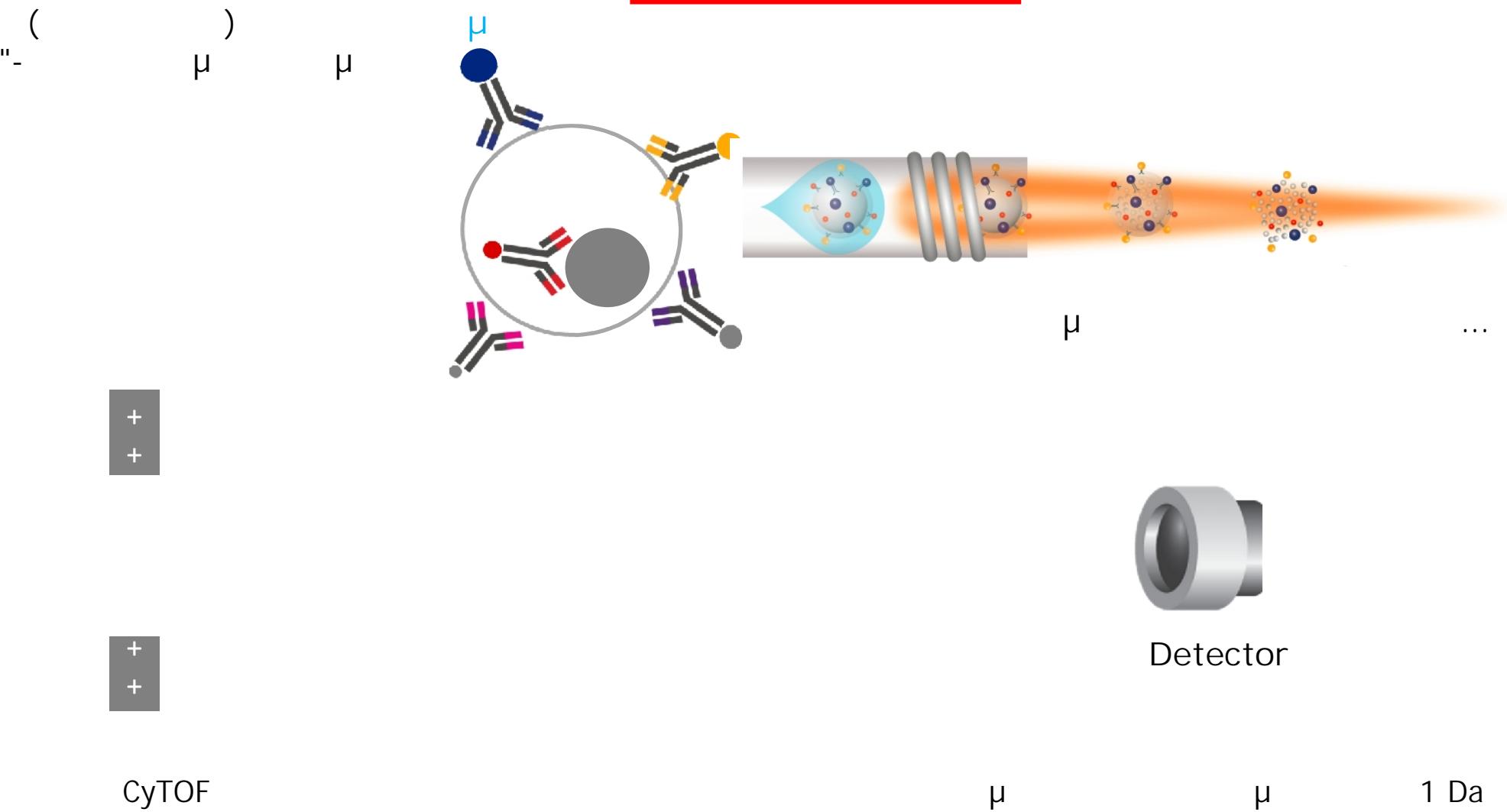
Analysis of multiparametric
data in mass cytometry (CyTOF)

Metals

Conjugated to
antibodies



Metal-tagged antibodies for specific protein targets are used to build high-dimensional panels for profiling cellular systems.

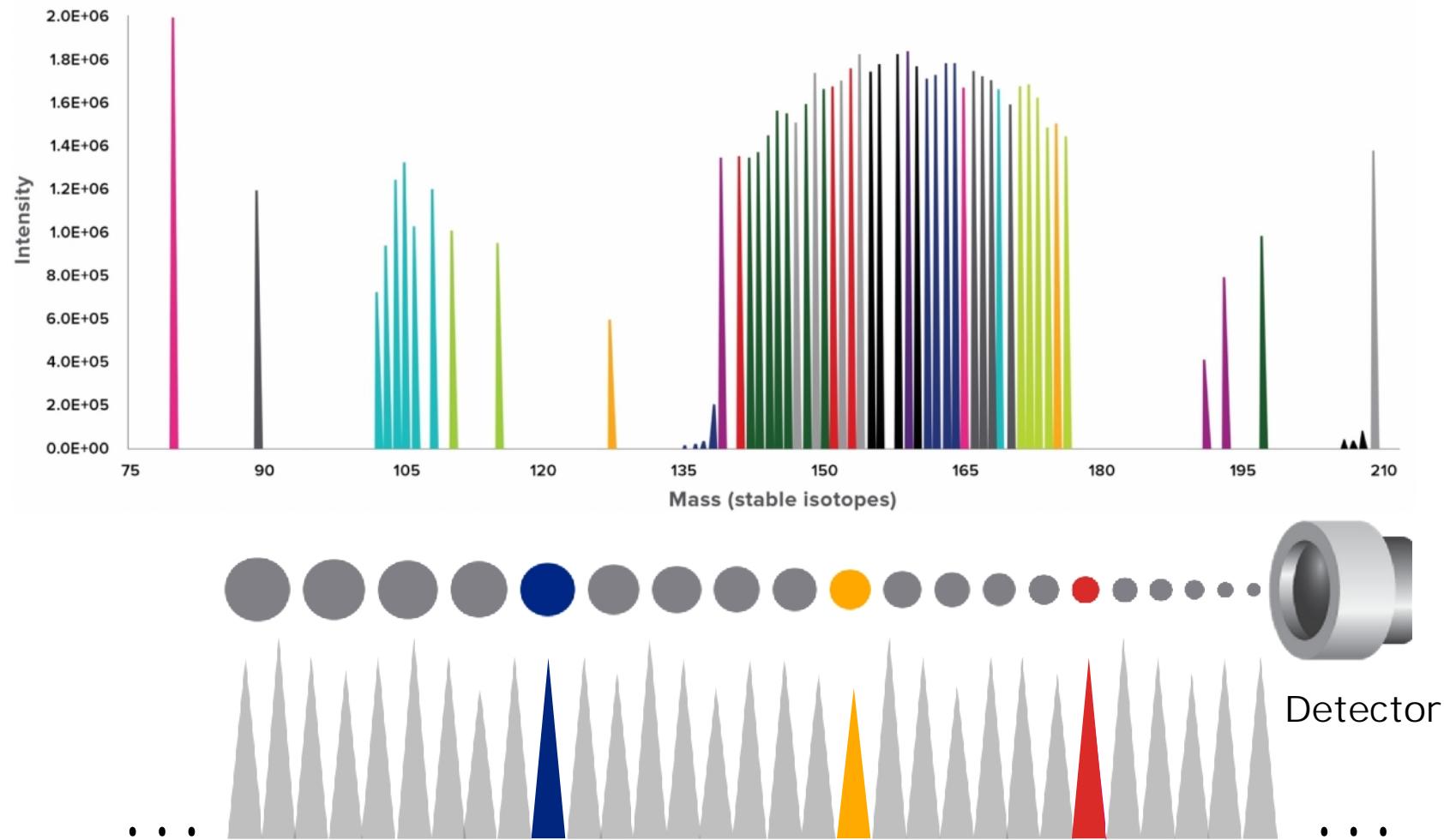


Heterogeneity in biology

Multiparameter cytometry
Colors and masses

Multiparameter cytometry
CyTOF - Principles

Analysis of multiparametric
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Heterogeneity in biology



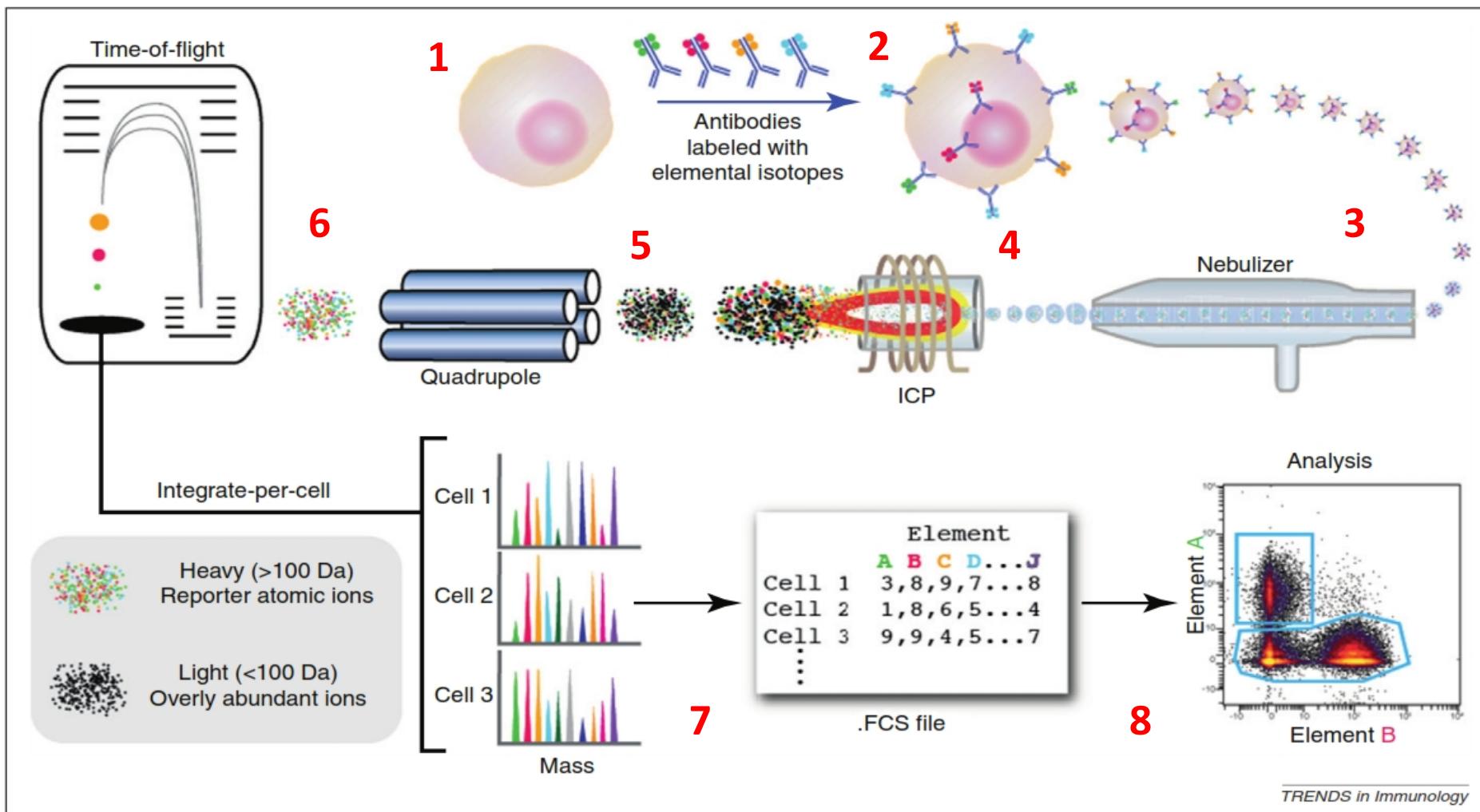
Multiparameter cytometry
Colors and masses



Multiparameter cytometry
CyTOF - Principles

Analysis of multiparametric
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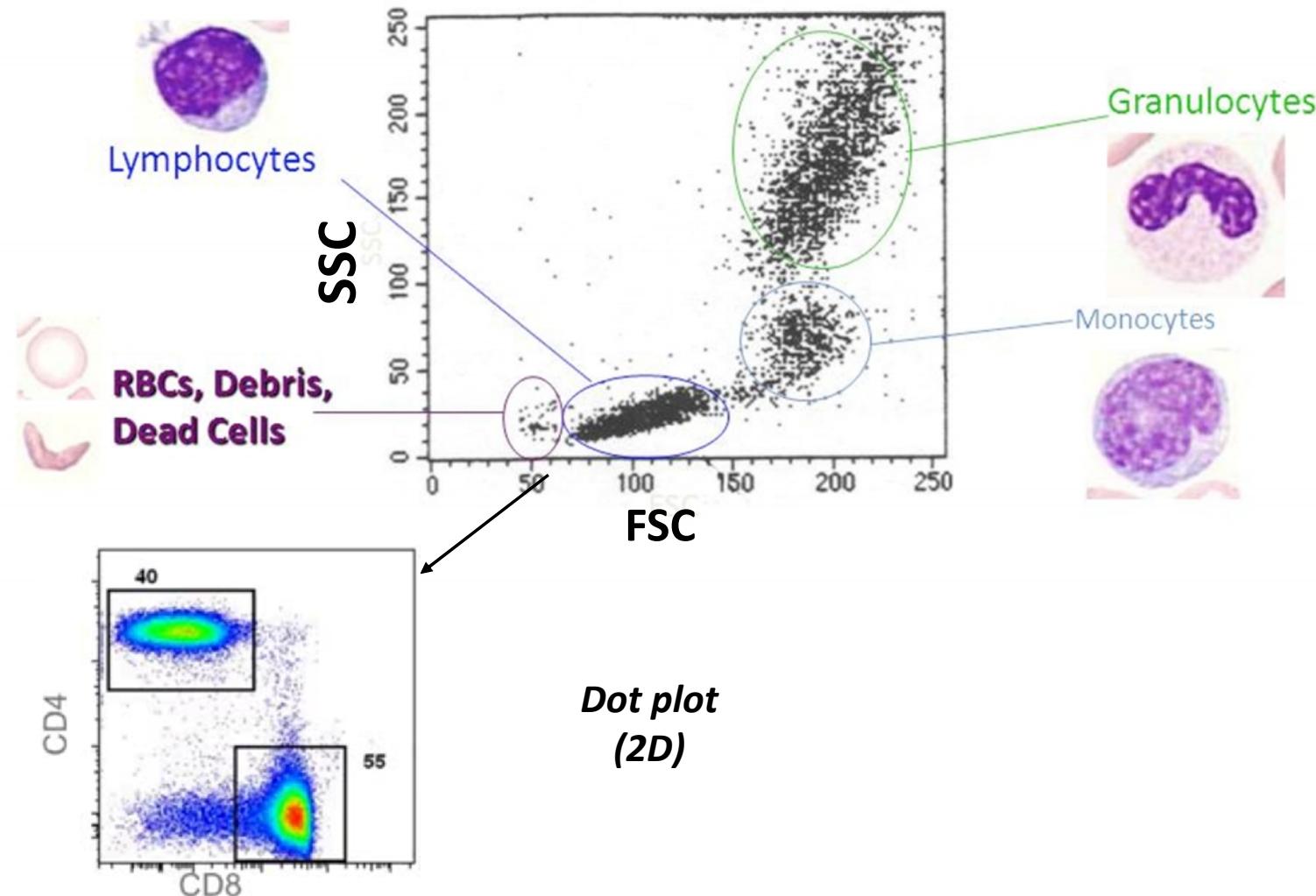


Heterogeneity in biology

Multiparameter cytometry
Colors and masses

Multiparameter cytometry
CyTOF - Principles

Analysis of multiparametric
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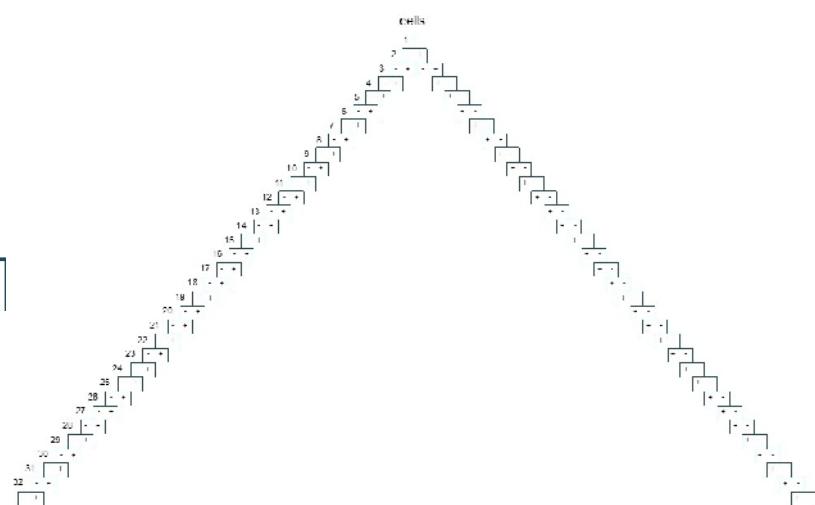
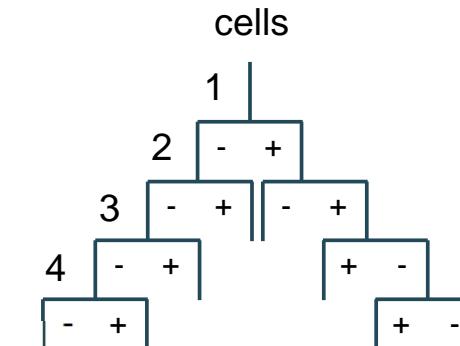
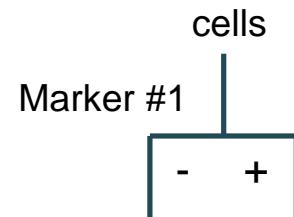


More Parameters = More Information

- 1 marker
- 2 possible phenotypes

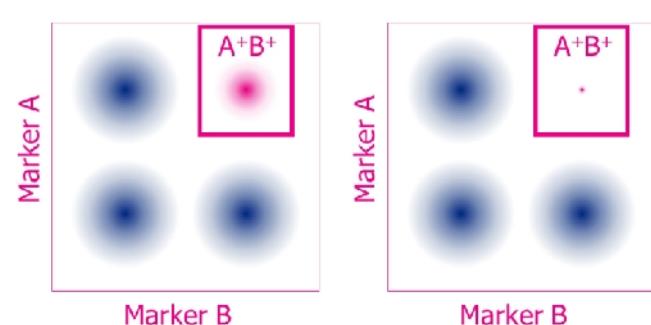
- 4 markers
- 16 possible phenotypes

32 markers
4.3 Billion



36 candidate markers
of the immune system:

A	B	C	D	E	F
G	H	I	J	K	L
M	N	O	P	Q	R
S	T	U	V	W	X
Y	Z	1	2	3	4
5	6	7	8	9	10



Heterogeneity in biology



Multiparameter cytometry
Colors and masses

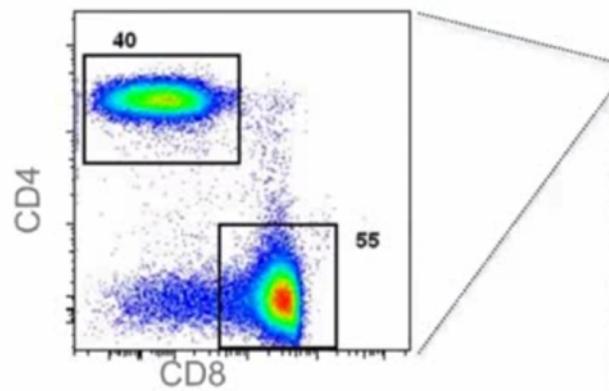


Multiparameter cytometry
CyTOF - Principles

Analysis of multiparametric
data in mass cytometry (CyTOF)

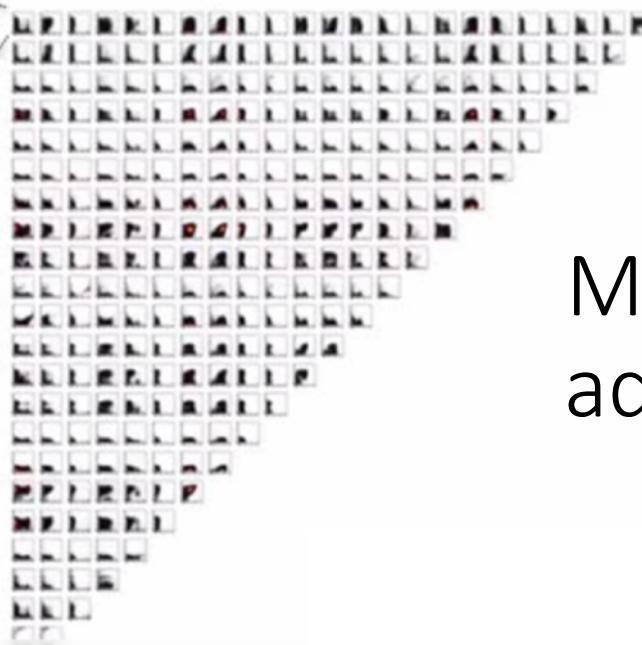


Two dimensional gating for traditional
cell populations is easy



But very difficult when multiple markers need
to be measured on a single sample

- T cell activation markers
- Checkpoint inhibitors
- Immune agonists



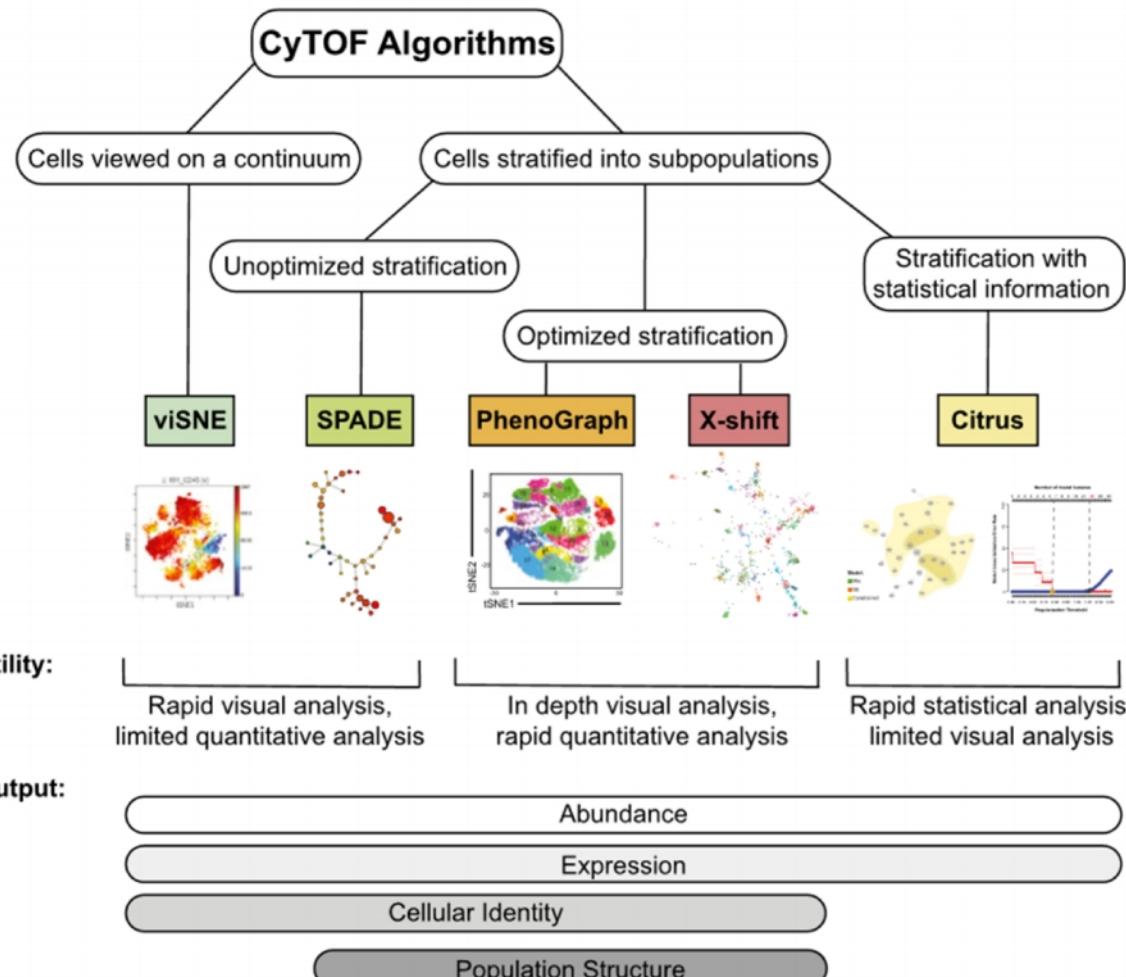
Machine Learning in
advanced cytometry

Heterogeneity in biology

Multiparameter cytometry
Colors and masses

Multiparameter cytometry
CyTOF - Principles

Analysis of multiparametric
data in mass cytometry (CyTOF)



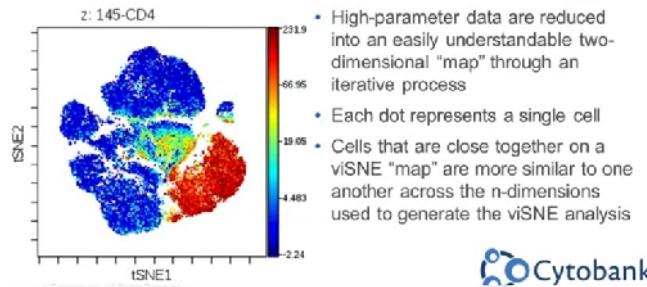
1. Exploratory maps
2. Dimensionality reduction
3. Clustering
4. Differential analysis

Kimball et al, JI, 2017

viSNE enables visualization¹ of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia

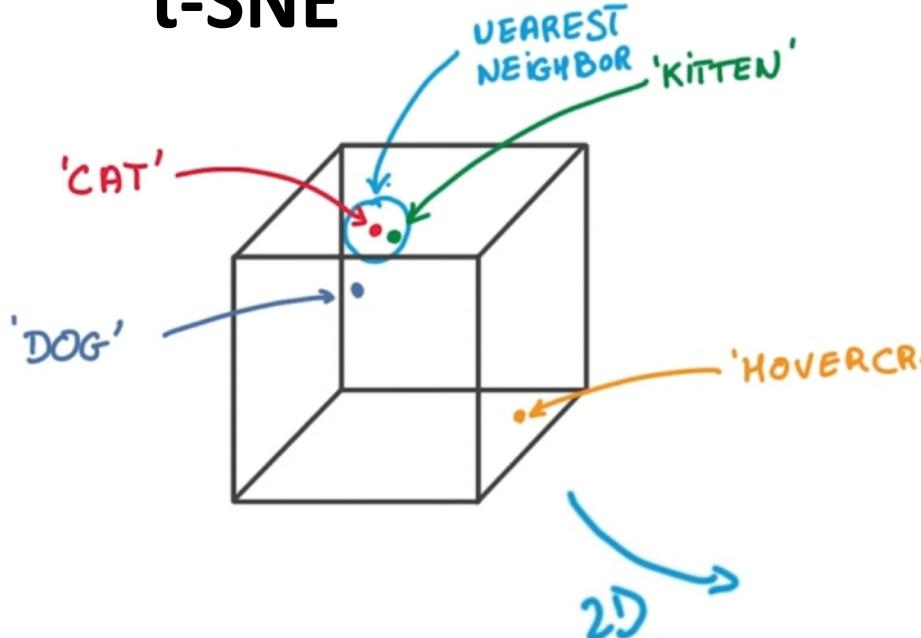
Elad David Amit^{1,2}, Kara L. Davis^{1,2}, Michelle D'Addario^{1,2}, Erin P. Simonds^{1,2}, Jacob H. Levine^{1,2}, Sean C. Bendall^{1,2}, Daniel K. Sheinfield^{1,2}, Amrit Krishnamoorthy¹, Garry P. Nolan^{1,2} & Dean Peter^{1,2}

NATURE BIOTECHNOLOGY VOLUME 31 NUMBER 6 JUNE 2013



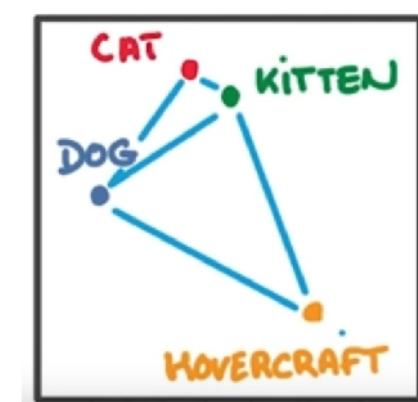
t-SNE

Cytobank

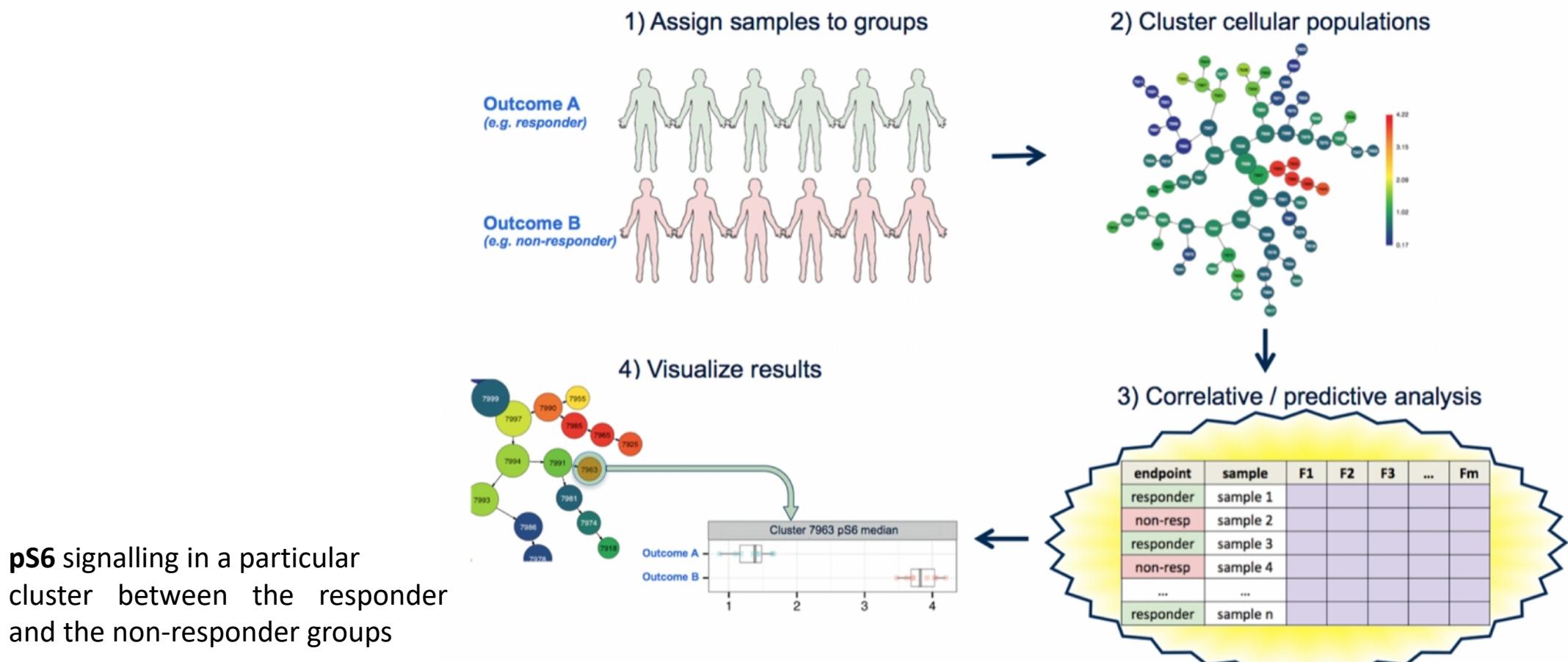


- Similar in high-dimensional space
- *shorter distance in two-dimensional space*

- Non-linear
- KL-Divergence



CITRUS: Cluster identification, characterization and regression



Heterogeneity in biology



Multiparameter cytometry



Colors and masses

Multiparameter cytometry



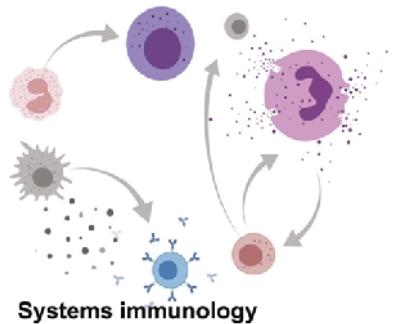
CyTOF - Principles

Analysis of multiparametric



data in mass cytometry (CyTOF)

COVID-19
Peripheral blood
Immunophenotyping



ICU vs non-ICU?

Disease severity



Maxpar Direct Immunophenotyping Assay (*Fluidigm*)



CD3	CD19	CD45	CD123	CCR7
CD4	CD20	CD45RA	CD127	CXCR3
CD8	CD25	CD45RO	CD161	CXCR5
CD11c	CD27	CD56	CD294	HLA-DR
CD14	CD28	CD57	CCR4	IgD
CD16	CD38	CD66b	CCR6	TCRγδ

E. Andreakos Lab, BRFAA

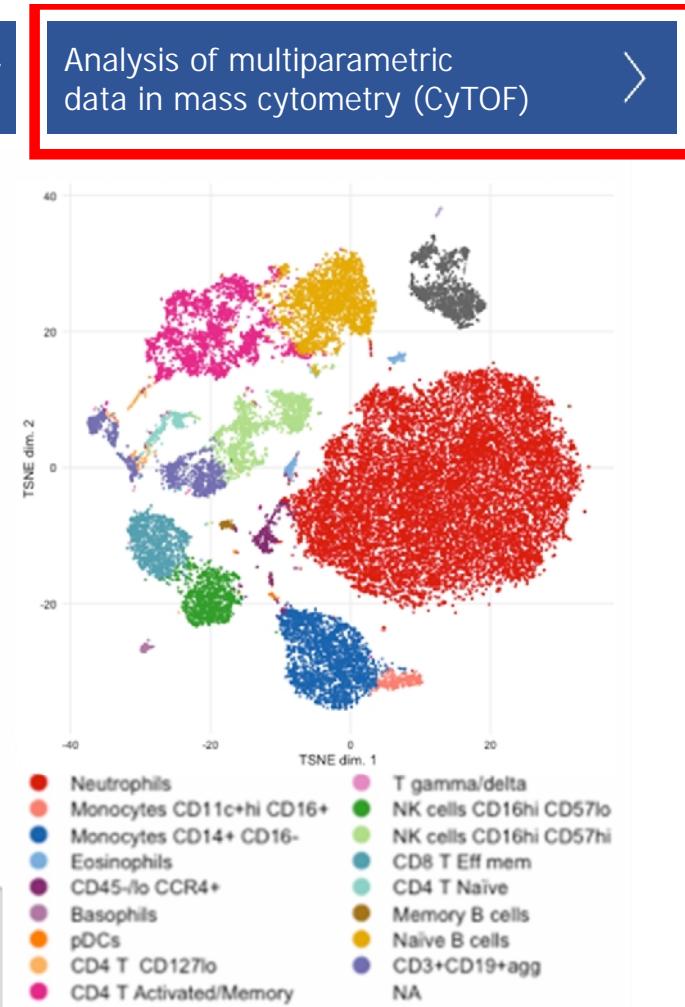
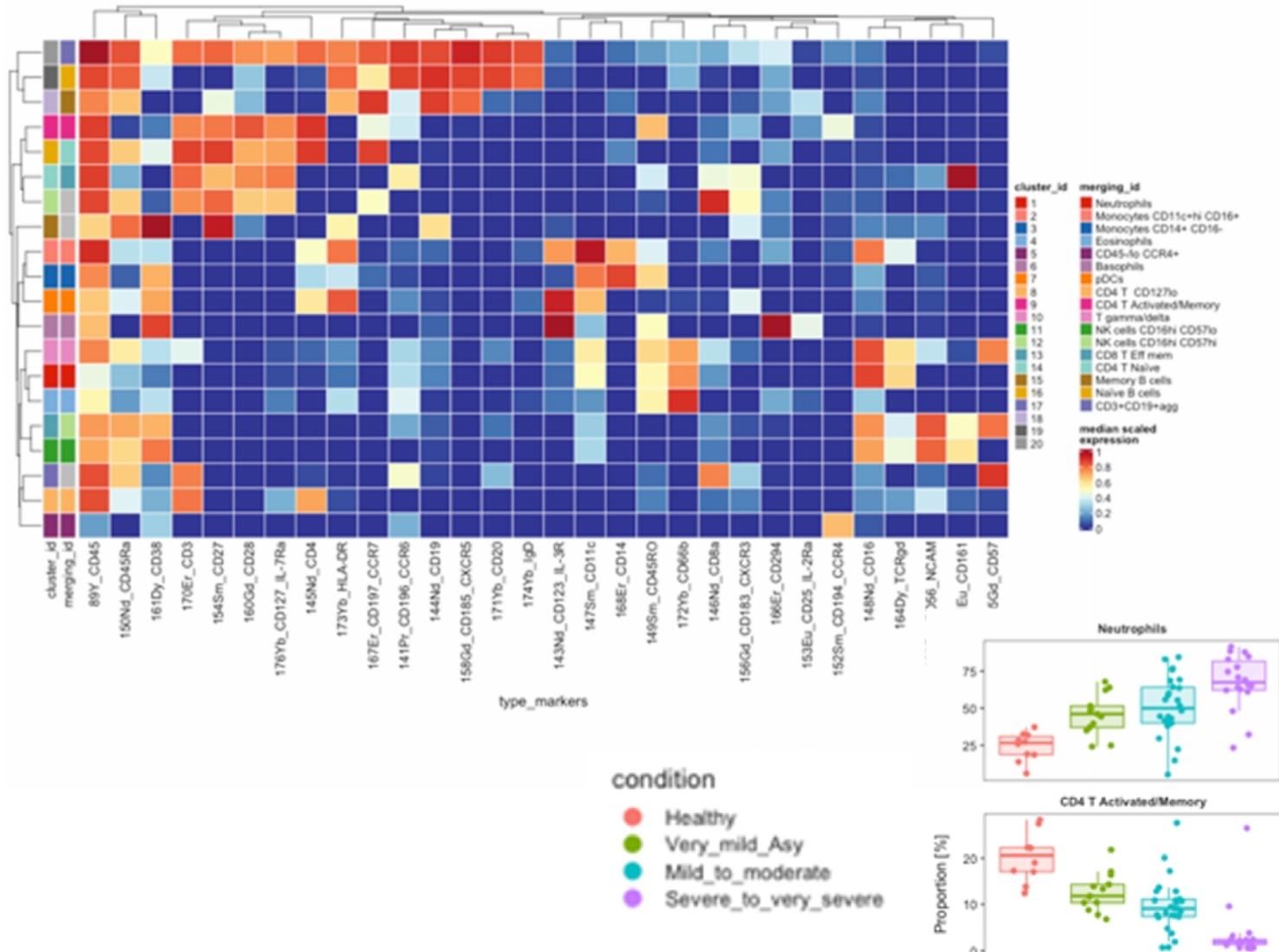
Papadaki et al, 2021 (manuscript in preparation)

Heterogeneity in biology

Multiparameter cytometry
Colors and masses

Multiparameter cytometry
CyTOF - Principles

Analysis of multiparametric
data in mass cytometry (CyTOF)



E. Andreakos Lab, BRFAA

Papadaki et al, 2021 (manuscript in preparation)

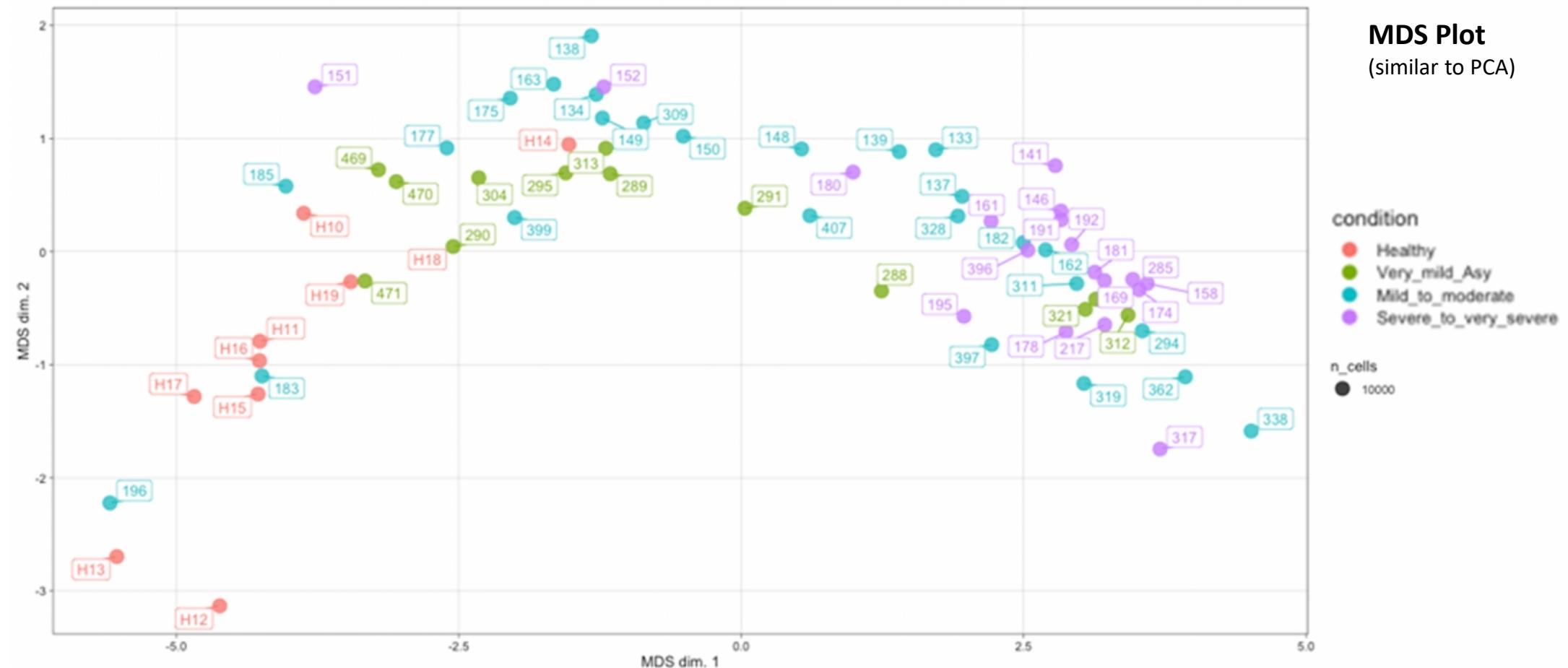
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MDS Plot
(similar to PCA)



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Papadaki et al, 2021 (manuscript in preparation)

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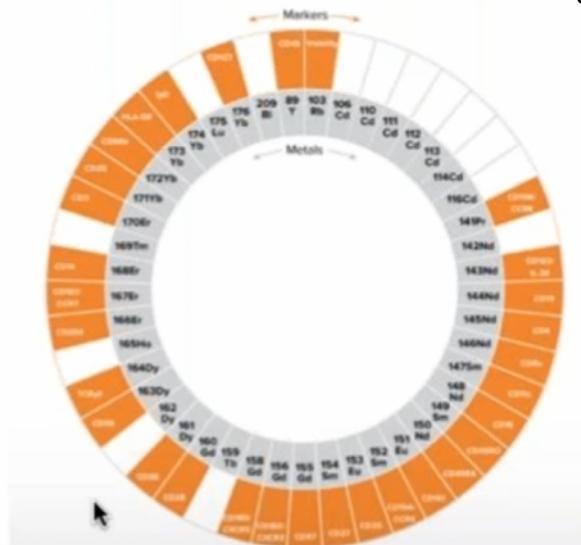
30-marker* panel with clones and metals

Antibody	Clone	Metal
CD45	HI30	^{89}Y
Live/dead indicator	N/A	^{103}Rh
CD196 (CCR6)	G034E3	^{141}Pr
CD123	6H6	^{143}Nd
CD19	HIB19	^{144}Nd
CD4	RPA-T4	^{145}Nd
CD8a	RPA-T8	^{146}Nd
CD11c	Bu15	^{147}Sm
CD16	3G8	^{148}Nd
CD45RO	UCHL1	^{149}Sm
CD45RA	HI100	^{150}Nd
CD161	HP-3G10	^{151}Eu
CD194 (CCR4)	L291H4	^{152}Sm
CD25	BC96	^{153}Eu
CD27	O323	^{154}Sm
CD57	HCD57	^{155}Gd

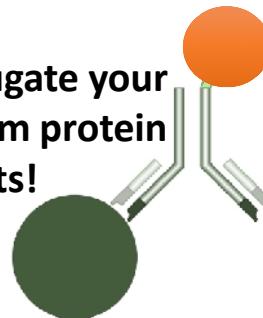
Antibody	Clone	Metal
CD183 (CXCR3)	G025H7	^{156}Gd
CD185 (CXCR5)	J252D4	^{158}Gd
CD28	CD28.2	^{160}Gd
CD38	HB-7	^{161}Dy
CD56 (NCAM)	NCAM16.2	^{163}Dy
TCR $\gamma\delta$	B1	^{164}Dy
CD294	BM16	^{166}Er
CD197 (CCR7)	G043H7	^{167}Er
CD14	63D3	^{168}Er
CD3	UCHT1	^{170}Er
CD20	2H7	^{171}Yb
CD66b	G10F5	^{172}Yb
HLA-DR	LN3	^{173}Yb
IgD	IA6-2	^{174}Yb
CD127	A019D5	^{176}Yb

*31 markers including the ^{103}Rh live/dead indicator

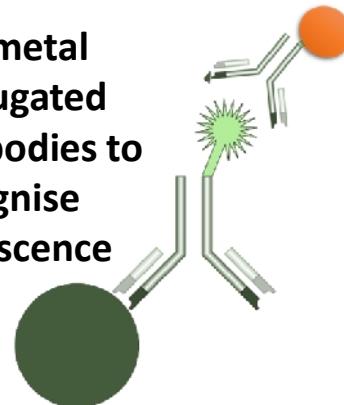
14 open channels[†] for panel customization



**Conjugate your
custom protein
targets!**



Use metal conjugated antibodies to recognise fluorescence



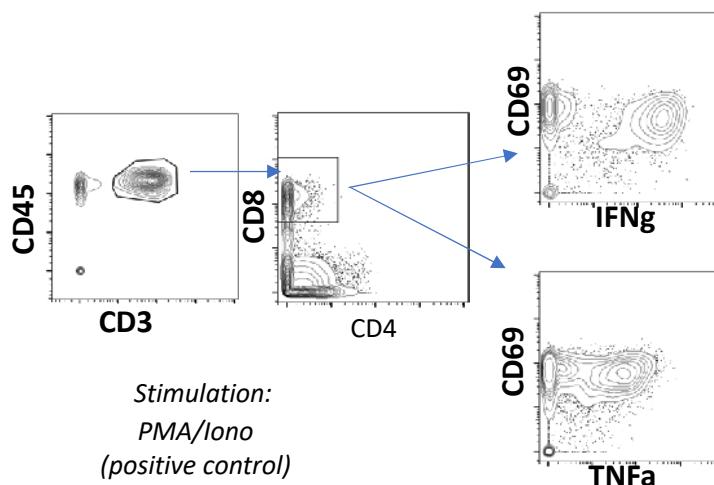
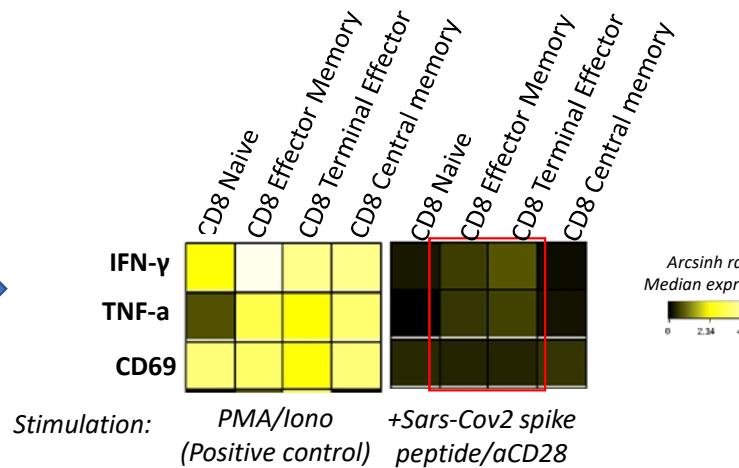
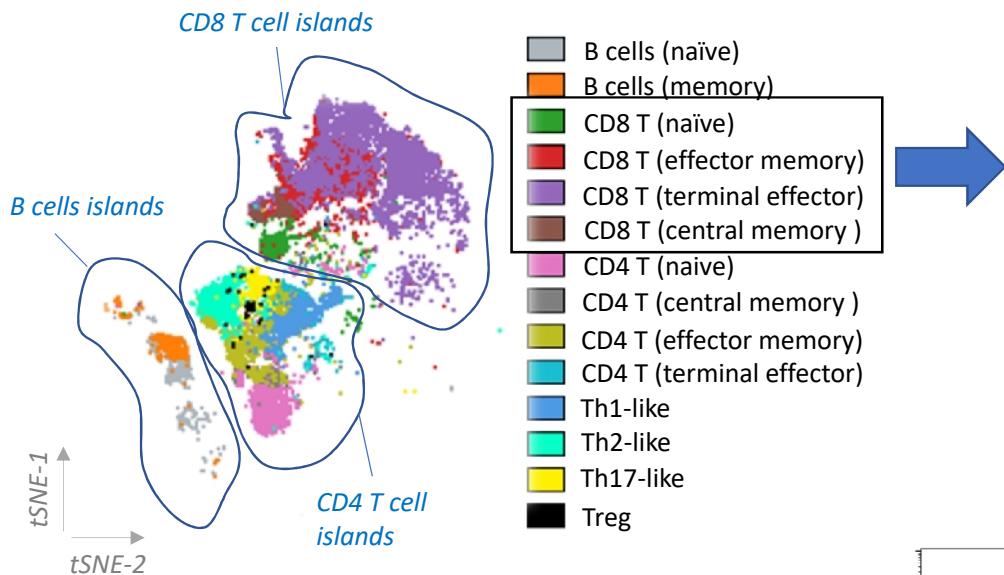
Heterogeneity in biology

Multiparameter cytometry
Colors and masses

Multiparameter cytometry
CyTOF - Principles

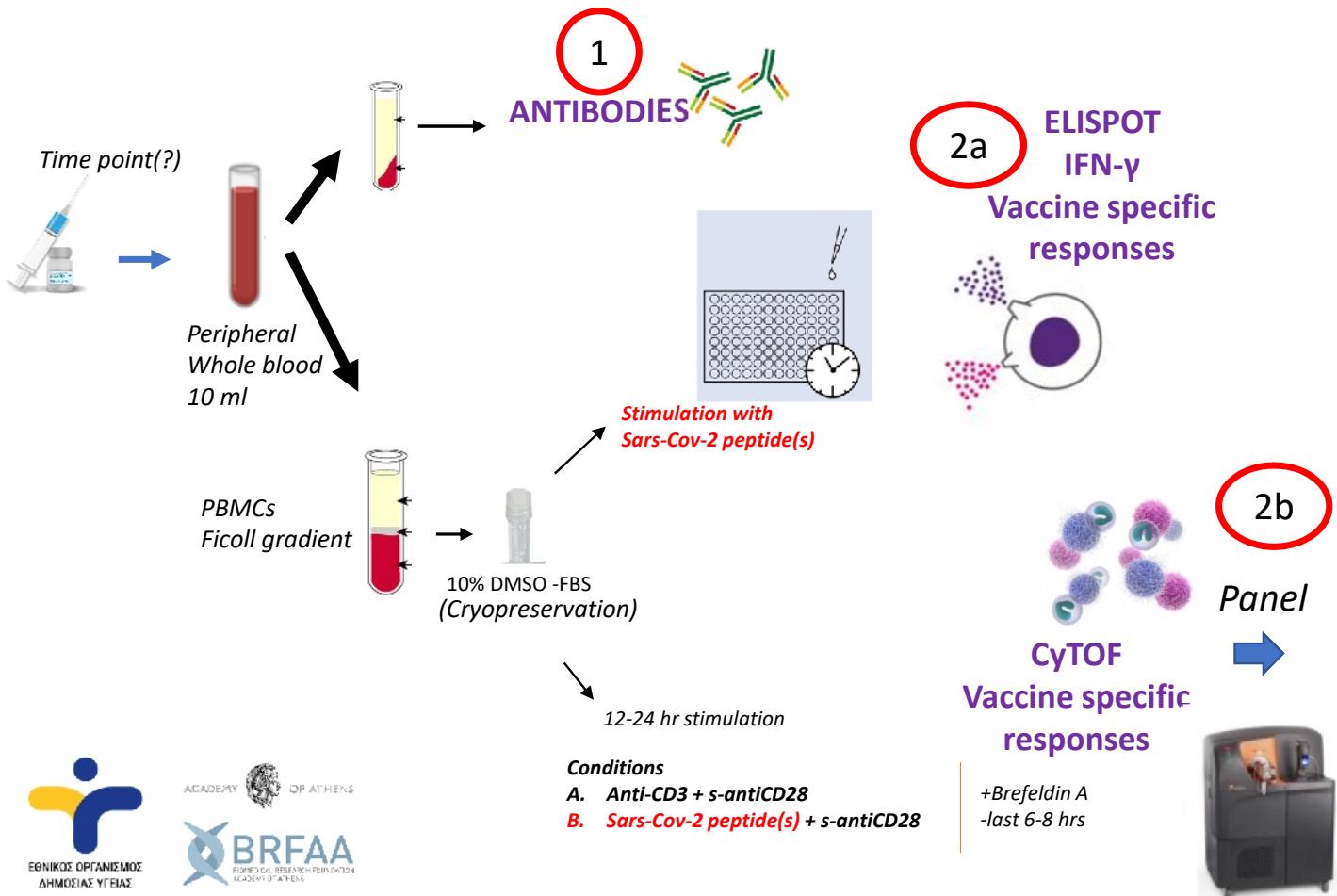
Analysis of multiparametric
data in mass cytometry (CyTOF)

PBMCs from Sars-CoV2 vaccinated donor
CyTOF analysis – Focus on lymphocytes
Panel: MDIPA + intracellular cytokines



Sars-Cov2 Vaccine efficacy and cellular responses

Aim: To determine antibody and T cell response profiles of COVID19-vaccinated and COVID19-recovered individuals



a/a	Target	Notes –target used for
1	CD45	Lineage
2	CD3	Lineage
3	CD4	Lineage
4	CD8	Lineage
5	CD19	Lineage
6	CD45RA	Memory profile of vaccine-induced T cells
7	CD45RO	Memory profile of vaccine-induced T cells
8	CCR7	Memory profile of vaccine-induced T cells
9	CD38	Kinetics of immune response/plasmablast frequency
10	CD20	Plasmablast frequency
11	CD27/CD28	Memory profile of vaccine-induced T cells
12	Ki-67	Kinetics of immune response
13	HLA-DR	Kinetics of immune response
14	CD25	Memory profile of vaccine-induced T cells
15	CD127	Memory profile of vaccine-induced T cells
16	Bcl-2	Kinetics of immune response
17	CD107a	Immunogenicity, polyfunctionality
18	Granzyme B	Immunogenicity, polyfunctionality
19	Perforin	Immunogenicity, polyfunctionality
20	CD40L	Immunogenicity, polyfunctionality (B cell help)
21	IFN- γ	Immunogenicity, polyfunctionality
22	IL-2	Immunogenicity, polyfunctionality
23	IL-4	Immunogenicity, polyfunctionality
24	IL-17	Immunogenicity, polyfunctionality

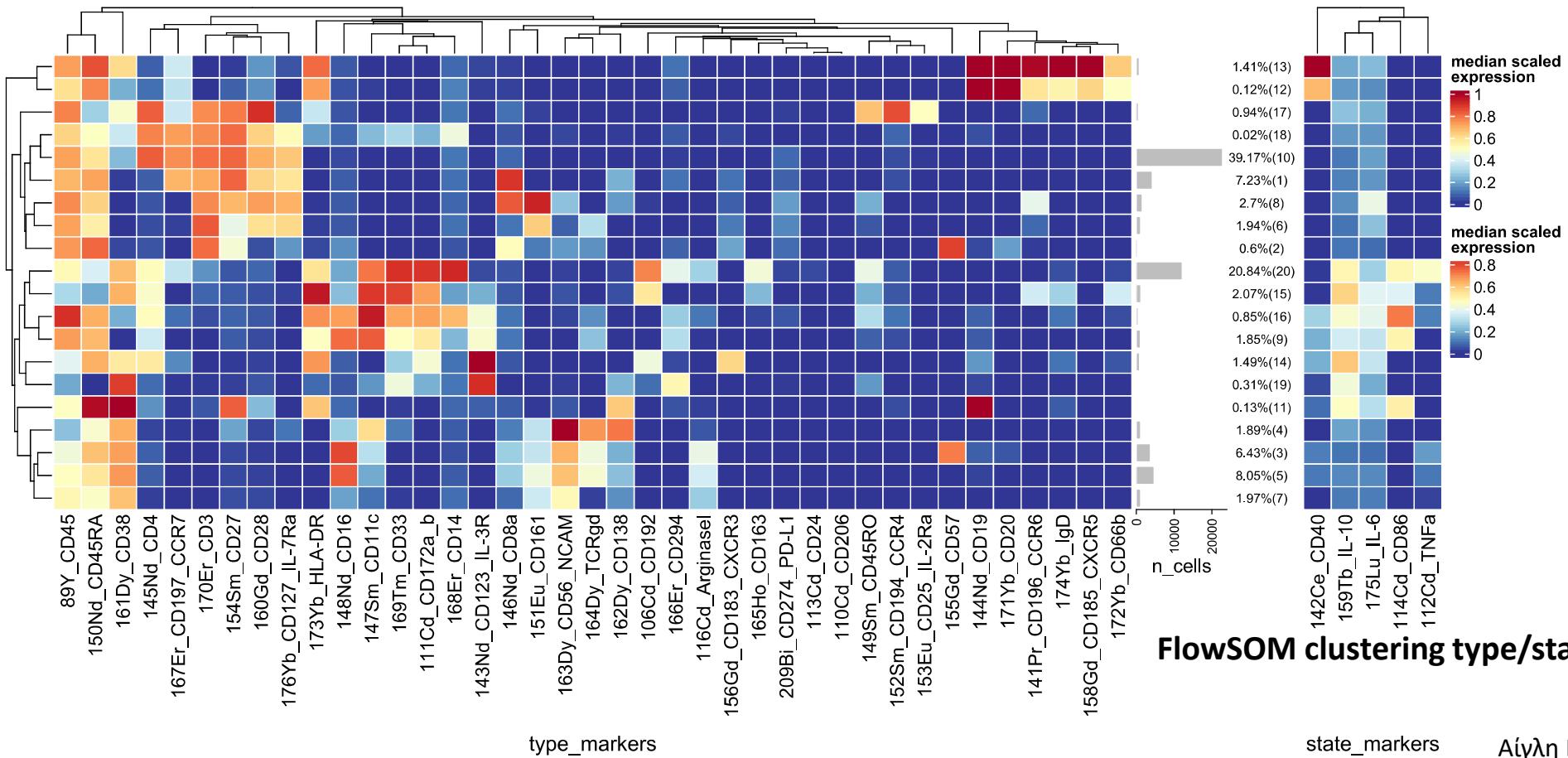
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Ανάλυση και σύγκριση ανοσοφαίνοτυπου περιφερικού αίματος διαφορετικών ομάδων ΣΚΠ (με διαφορετικά κλινικά χαρακτηριστικά) + προσθήκη δεικτών ενεργοποίησης π.χ. κυταροκίνες



Ευχαριστώ για την προσοχή σας!

Πηγές, more info και επικοινωνία!

- Lab website → <https://www.brfaacytoflab.com>
- Check Scott Taner, Gary Nolan, Sean Bendall, Bernd Bodenmiller
- Nice analysis guide! → Kimball et al 2017, Journal of Immunology
- Cytobank → <https://cytobank.org/>
- Pathsetter → <https://www.fluidigm.com/singlearticles/pathsetter>
- Nowicka M, Krieg C, Crowell HL *et al.* CyTOF workflow: differential discovery in high-throughput high-dimensional cytometry datasets [version 3; peer review: 2 approved]. *F1000Research* 2019, **6**:748 (<https://doi.org/10.12688/f1000research.11622.3>)

CyTOF Lab - IIBEAA

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Services

